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ABSOLUTE CONCENTRATIONS OF DITHRANOL AND TRIACETYL-DITHRANOL IN THE SKIN LAYERS AFTER LOCAL TREATMENT: IN VIVO INVESTIGATIONS WITH FOUR DIFFERENT TYPES OF PHARMACEUTICAL VEHICLES

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The pharmacokinetics of dithranol (anthralin) and its triacetate were investigated by employing a method which determines the quantity of the drug that penetrated into single layers of the human skin in vivo. For this purpose, tritium-labeled dithranol or triacetyl-dithranol was incorporated into four different ointments. The ointments were applied to the skin and biopsies were taken after 10, 30, 100, and 1000 min. The horny layer was removed before biopsy by Scotch tape stripping. The biopsies were sliced horizontally and the tritium determined in each sample.

Dithranol as well as its triacetate penetrated best from more hydrophobic ointments (Vaseline and aqueous wool-wax-alcohol ointment). From hydrophilic ointments (polyethylene glycol ointment and aqueous hydrophilic cream), only poor penetration was observed. Dithranol penetrated in far greater amounts than its triacetate, and the two compounds revealed fundamentally different penetration kinetics in epidermis and dermis. The data indicate that the triacetate was not split into its parent compound, dithranol, in substantial quantity, as the data obtained show the criteria of two independent substances.

The penetration of drugs into the skin after local application is an extremely complicated process. Experimental approaches to the processes can follow two paths: (1) The total skin can be considered as a physical membrane and the penetration of substances through this membrane can be observed. This allows generalized conclusions about the penetration process, but yields no information about absolute concentrations of drugs in the skin at a given time. (2) The penetration process in various compartments of skin can be measured. In this case, valid statements can be made only for a definite substance under set conditions. Each alteration in experimental conditions (method of application, concentration of substance in the ointment, conditions at site of application) can lead to alterations in any of the compartments and so influence the results.

The latter approach is of particular importance in questions arising in connection with the external therapy of dermatoses, if one requires to know the concentrations of a selected drug in a definite skin layer at a fixed time after application. We undertook investigations of the penetration kinetics of a series of drugs presently applied or under consideration in external therapy under conditions as near as possible to those in clinical situations. We chose to study dithranol, an agent whose clinical effects are well documented but whose mechanism of action remains controversial. A series of hypotheses exist for the mechanism of action of this substance based on observations of biochemical, molecular biologic, and morphokinetic effects of the drug in experimental investigations. Exact estimations of concentrations in different skin layers after therapeutic applications could narrow the selection from these hypotheses. Only those effects observed at concentrations at or below those found in vivo in the skin would be relevant. Furthermore, a derivative of dithranol, triacetyl-dithranol, has been considered as an antipsoriatic agent [1]. An hypothesis was developed whereby the acetyl groups are split off by the skin esterases so that dithranol is liberated. In this way, the disadvantages of dithranol (discoloration and inflammatory irritation) would be obviated. Initially, we had to investigate whether and in what concentrations triacetyl-dithranol penetrates the skin.

MATERIALS AND METHODS

Dithranol (anthra-1,8,9-triol) was purchased from Bayer, Leverkusen, West Germany, and triacetyl-dithranol (triacetyl-anthra-1,8,9-triol) from Knoll AG, Ludwigshafen, West Germany. These substances were labeled with tritium (dithranol by a base-catalyzed unspecific exchange with tritiated water, and triacetyl-dithranol by platinum-catalyzed unspecific exchange in tritiated acetic acid) by Radiochemicals Amersham,

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England, with a specific activity of 2.3 mCi/mg each, purified by thin-layer radiochromatography and the radioactive purity controlled monthly. Batches with more than 5% impurity were repurified. Exchange of labile tritium against hydrogen in water was checked and found to be less than 1% per 24 hr at room temperature. Labeled substances were mixed with unlabeled drug and made up with the 4 ointment bases of the German Pharmacoepia (DAB 7) at 0.1% concentration (approximately 0.2 μCi/mg ointment). The carriers used were: (1) a fat-like hydrocarbon mixture (white Vaseline); (2) a hydrophobic water-in-oil (w/o) emulsion (aqueous wool-wax-alcohol ointment); (3) a hydrophobic oil-in-water (o/w) emulsion (aqueous hydrophilic ointment); and (4) a hydrophilic polyethylene glycol mixture (polyethylene glycol ointment).

Ointment (70–100 mg) was rubbed for 50 sec into a 28-cm^2 area of intact human skin. The patients were mainly women (37 female, 3 male) 40 years of age or more. In order to avoid spreading of ointment, the skin was covered with a wire cage until excision. The ointment was left on the skin for periods of 10, 30, 100, 300, and 1000 min. The residue was carefully washed off several times by gentle cleaning with cotton and isopropanol and the horny layer stripped off using cellotape strips (15 ± 5 times). The horny layer was considered to be 20 μm thick. A piece of skin of at least 8 mm diameter was then excised. A 28-mm^2 sample of the preparation was sectioned horizontally using a freezing microtome. In control specimens the thickness of the epidermis averaged 160 μm (100–200 μm). The underlying layer, the thickness of which averaged 1050 μm, was regarded as dermis. The first 16 sections (10 μ thick) were therefore termed "epidermis," and the following 25 sections "dermis."

The radioactivity of the horny layer on the cellotape was measured using a liquid-scintillation counter. Since the sticky layer of the cellotape dissolves in the scintillation fluid, the same mixture (0.8% butyl-PBD; 5% Biosolv, Beckman) in toluene could be used for horny layer as well as dermis and epidermis. The tissue slices were left for 6 hr at 70°C in 0.3 ml of NaOH solution (0.5 N) and were neutralized with acetic acid before addition of scintillation mixture. The measurements (dpm) were calculated as percentage and as absolute concentrations for the various layers. The amount of substance in the several layers (e.g., horny layer, epidermis, dermis) per unit area of tissue is represented as $\mu g \cdot cm^{-2}$; $\mu g \cdot ml^{-1}$ represents the amount of substance in a unit volume of 1 ml; and μmolar indicates the moles of substance in a unit volume of 1 liter of the tissue.

RESULTS

Figures 1 and 2 display the penetration kinetics of dithranol and triacetyl-dithranol in intact human skin (in vivo) and allowed the following conclusions:

1. Dithranol and triacetyl-dithranol have two completely different types of penetration kinetics.

2. The penetration of both substances was considerably influenced by the carrier. Lipophilic ointment bases, particularly the single-phase base Vaseline, allowed the best penetration of both drugs into the epidermis.

3. The maximal amount penetrating into the epidermis (160 μ depth) was 0.3 μg/cm^2 skin surface for dithranol and 0.04 μg/cm^2 for triacetyl-dithranol in Vaseline.

4. The maximal dermal concentrations were in reversed relationship: 0.1 μg/cm^2 for triacetyl-dithranol and 0.045 μg/cm^2 for dithranol in Vaseline.

Tables I and II show the molar concentrations which can be compared with biochemical and pharmacologic data. Within 300 min, dithranol concentrations of almost 0.1 mmolar may be reached in the epidermis when Vaseline is used as base, whereas the epidermal concentration of triacetyl-dithranol does not exceed 0.01 mmolar. Different bases influence the distribution of the drugs within skin layers and in respect to the application time in a different manner. This behavior is modified by the drug itself and no general rules can be derived. Particularly noteworthy is that the usual reservoir function of the horny layer no longer pertains with dithranol, particularly with lipophilic ointment base. In comparison to many other substances, a very high concentration of dithranol is found in the epidermis with very low levels in the horny layer and dermis (Figs. 3, 4). This is not the case with triacetyl-dithranol where the horny layer reservoir function applies.

The quantity of dithranol in the epidermis in

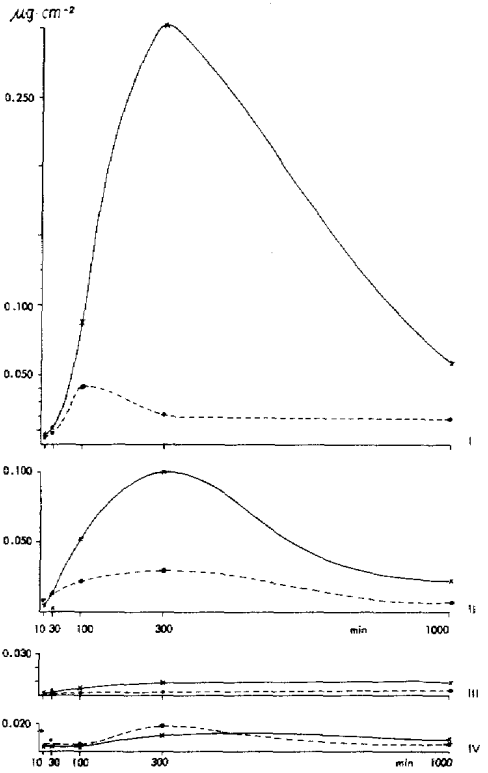

FIG. 1. Influence of different bases on penetration kinetics of dithranol ($\mu g \cdot cm^{-2}$ skin surface) in the human epidermis (×——×) and dermis (●-----●) in vivo. *I* = Vaseline; *II* = aqueous wool-wax-alcohol ointment; *III* = aqueous hydrophilic ointment; *IV* = polyethylene glycol ointment. Penetration time: 10–1000 min.

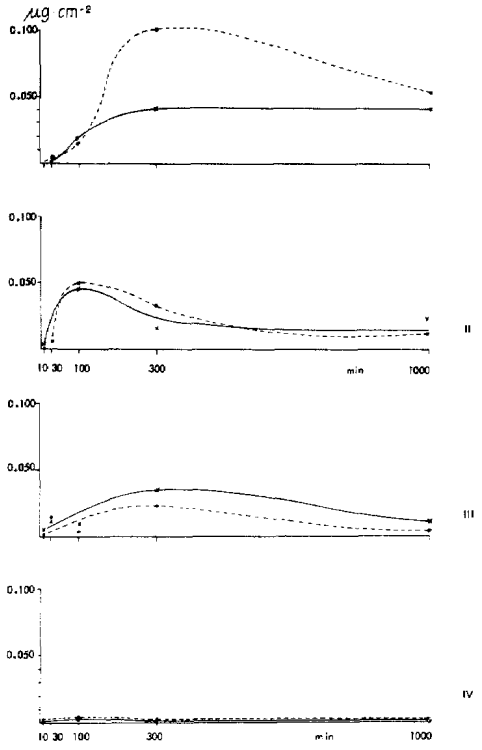

FIG. 2. Influence of different bases on penetration kinetics of triacetyl-dithranol ($\mu g \cdot cm^{-2}$ skin surface) in the human epidermis and dermis. *I* = Vaseline; *II* = aqueous wool-wax-alcohol ointment; *III* = aqueous hydrophilic ointment; *IV* = polyethylene glycol ointment.

TABLE I. *Micromolar concentrations of dithranol after 30-min and 300-min application time*

Skin layer	Penetration time (*min*)	Vaseline	Aqueous wool-wax-alcohol ointment	Aqueous hydrophilic ointment	Polyethylene glycol ointment
Horny layer	30′	9.91	115.6	22.05	39.67
	300′	32.49	120.7	22.51	138.7
Epidermis	30′	2.81	0.77	0.79	1.10
	300′	93.66	30.44	2.65	3.58
Dermis	30′	0.36	0.51	0.041	0.195
	300′	0.91	1.13	0.034	0.72

percent of the applied dose decreases with increasing water content or hydrophilic properties of the ointment base (Vaseline → w/o emulsion → o/w emulsion → polyethylene glycol) (Fig. 3). Triacetyl-dithranol concentrates in the dermis when a lipophilic base is used (after 300 min with Vaseline, after 1000 min with aqueous wool-wax-alcohol ointment), the dermal values with Vaseline being considerably higher than the concentrations found in the epidermis. The application of hydrophilic ointment bases is pointless for either substance. The following advantages are, however, noticeable: (1) Triacetyl-dithranol in o/w emulsions penetrates just as well into the epidermis as does the same medication with Vaseline. (2) Dithranol penetrates from both hydrophilic carriers in very small but constant amounts (approximately 0.01 $\mu g/cm^2$) into the epidermis. The application of a hydrophilic single-phase base (polyethylene glycol) brings about a high depot in the horny layer for both substances. Further penetration into the epidermis and dermis was almost completely inpeded in the case of triacetyl-dithranol and considerably diminished for dithranol (Figs. 1, 3).

DISCUSSION

The antipsoriatic effect of dithranol is proved and noncontroversial, although the mechanism of action has been only partially defined. Generally accepted proof has been forthcoming with respect to its cytostatic properties [2] and inhibition of RNA-polymerase [3] as well as other enzymes [4]. As is usual with in vitro studies, the question has been posed as to what extent the results are valid for living skin. Thus, it must be ascertained to what extent dithranol penetrates to the various skin layers under therapeutic conditions.

Based on fluorescence studies [5], it was hypothesized that dithranol has a cytotoxic effect in the first layer of prickle cells after transversing the horny layer. Following this, dithranol is immediately transformed. The inflammation concomitant with dithranol therapy can be considered to be the result of this cell destruction which is followed in the repair stage by the development of orthokeratosis. According to this hypothesis, the cytostatic effects of dithranol are of no importance as the substance itself never reaches the basal cells in cytostatic quantities. These contradictory hypotheses provoked us to investigate the distribution of dithranol in the skin after local application and to discover which effects are possible in the individual skin layers based on the quantity that penetrates. A dependence of penetration on the carrier is to be expected from previous studies [6–12]. Dithranol is usually applied in Vaseline because this fat-like hydrocarbon meets both certain therapeutic requirements in psoriasis as well

TABLE II. *Micromolar concentration of triacetyl-dithranol after 30-min and 300-min application time*

Skin layer	Penetration time (*min*)	Vaseline	Aqueous wool-wax-alcohol ointment	Aqueous hydrophilic ointment	Polyethylene glycol ointment
Horny layer	30′	142.80	176.16	309.13	36.49
	300′	196.33	265.35	170.16	70.81
Epidermis	30′	0.300	5.01	2.32	0.28
	300′	7.42	2.32	6.43	0.124
Dermis	30′	0.068	0.190	0.378	0.057
	300′	3.03	0.876	0.61	0.025

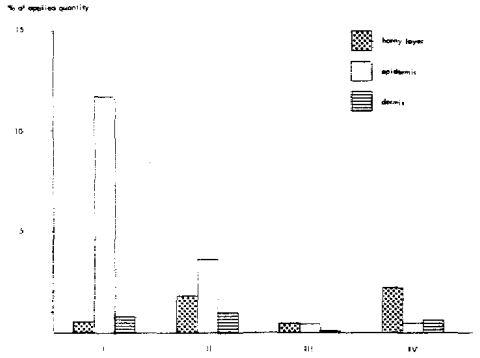

FIG. 3. Quantity of dithranol determined in the skin in vivo in percent of the applied amount (bases as in Fig. 1).

as the pharmaceutical requirement of stability of the substance in the carrier. In the presence of water, dithranol undergoes decomposition.

The present investigations show that dithranol penetrates into the epidermis in large quantities with Vaseline. The horny layer does not function as either a reservoir or a penetration barrier [13]. This unusual behavior requires particular mention. The chemical characteristics of the molecule are similar to known substances with good penetrating properties. On the one hand, it possesses a high dipole moment due to the unilateral projecting oxygen atoms, similarly to dimethylsulfoxide, dimethylformamide, acetone, and also water. On the other hand, hydrogen bonds can form as seen with salicylic acid. This property is most distinct in the absence of water since the attachment of water, present in the outer phase of an emulsion, to the dithranol molecule will reduce its dipole moment so that the penetration capacity of dithranol through the horny layer would be reduced. Polyethylene glycol, a long-chain polar molecule will similarly lower the penetration of dithranol. After application of aqueous hydrophilic ointments and particularly the hydrophilic carrier, polyethylene glycol, a certain horny layer depot effect is found. The penetration rate is simultaneously considerably reduced. This indicates that the liberation of the drug from the base, stored in the horny layer, is diminished.

If the free hydrophilic groups of dithranol are blocked (e.g., esterified with acetic acid in triacetyl-dithranol) then, as expected, the physicochemical properties of dithranol are altered and as a consequence the extremely rapid penetration through the human skin is lost. There is no reason to assume an active transport in the skin. The fact that the concentrations of dithranol in the epidermis are higher than in the horny layer therefore reflects very clearly different physicochemical properties of these two layers. A partition of the drug between two phases takes place where one phase, the epidermal tissue, has a far greater affinity for the substance than does the horny layer. Together with the reduced barrier function of the horny layer, this behavior indicates a considerably high absorption rate of dithranol. It should be emphasized, however, that the largest portion of an ointment and its ingredients remains on the surface of the skin as can be seen from Figures 3 and 4.

The high rate of penetration of dithranol into the horny layer from Vaseline with a correspondingly very high concentration in the epidermis has important consequences. Concentrations of between approximately 1 and 100 μmolar were observed in the epidermis after a penetration time of 300 min (Tab. I). This means that 10^{-6} to 10^{-4} moles per liter of epidermal tissue are found. These concentrations are cytotoxic, since the most important metabolic pathways of the cells (the respiratory chain and glycolysis) are inhibited [2].

Since dithranol does not dissolve in water in the concentrations to be found in the epidermis, one must assume that dithranol is bound to protein. Perhaps some dithranol is converted to water-soluble product in the epidermis. This question of metabolism should be further investigated by thin-layer chromatography. The amounts found do not preclude that sufficient quantities of unaltered dithranol reach the stratum germinativum in cytostatic levels. It seems not necessary to accept mitosis inhibition as the exclusive mechanism of action.

The esterification of dithranol with acetic acid and its therapeutic application in psoriasis is based, as mentioned, on the advantage of a colorless transport molecule which is supposed to penetrate easily through the horny layer to the epidermis. The results presented show that this does not occur at a perceptible rate, but rather that the two compounds have different penetration kinetics.

The function of the horny layer as a reservoir and barrier is completely upheld in the case of triacetyl-dithranol. The quantities penetrating into the epidermis are fairly small; on the other hand, considerable quantities gradually concentrate in

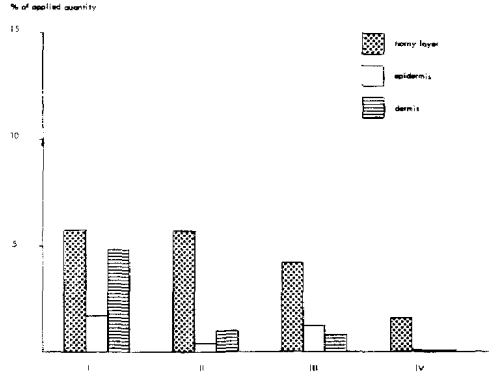

FIG. 4. Quantity of triacetyl-dithranol determined in the skin in vivo in percent of the applied amount (bases as in Fig. 1).

the dermis. This different behavior of triacetyl-dithranol relative to dithranol allows us to conclude that the former substance is not transformed in the skin to dithranol or its metabolites.

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